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THREE HIERARCHIES IN SKELETAL MUSCLE FIBRE CLASSIFICATION ALLOTYPE, ISOTYPE AND PHENOTYPE[1]

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ABSTRACT Immunocytochemical analyses using specific anti-myosin antibodies of mammalian muscle fibres during regeneration, development and after denervation have revealed two distinct myogenic components determining fibre phenotype. The jaw-closing muscles of the cat contain superfast fibres which express a unique myosin not found in limb muscles. When superfast muscle is transplanted into a limb muscle bed, regenerating myotubes synthesize superfast myosin independent of innervation. Reinnervation by the nerve to a fast muscle leads to the expression of superfast and not fast myosin, while reinnervation by the nerve to a slow muscle leads to the expression of a slow myosin. When limb muscle is transplanted into the jaw muscle bed, only limb myosins are synthesized. Thus jaw and limb muscles belong to distinct allotypes, each with a unique range of phenotypic options, the expression of which may be modulated by the nerve. Primary and secondary myotubes in developing jaw and limb muscles are observed to belong to different categories characterized by different patterns of myosin gene expression. By taking into consideration the pattern of myosins synthesized and the changes in fibre size after denervation, 3 types of primary (fast, slow and intermediate) fibres and two types of secondary (fast and slow) fibres can be distinguished in rat fast limb muscles. All primaries synthesize slow myosin soon after their formation, but this is

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withdrawn in fast and intermediate primaries at different times. After neonatal denervation, slow and intermediate primaries express slow myosin whereas fast primaries do not, and slow primaries hypertrophy while other fibres atrophy. In the mature rat, the number of slow fibres in the EDL is less than the number of slow primaries. Upon denervation, hypertrophic slow fibres matching the number and topographic distribution of slow primaries appear, suggesting that a subpopulation of slow primaries acquire the fast phenotype during adult life, but reveal their original identity as slow primaries in response to denervation by hypertrophying and synthesizing slow myosin. It is proposed that within each muscle allotype, the various isotypes of primary and secondary fibres are myogenically determined, and are derived from different lineages of myoblasts.

INTRODUCTION

Fibres of limb and trunk muscles of mammals have been classified phenotypically into slow, fast-red and fast-white types, each type containing a distinct type of myosin, and associated with a specific profile of metabolic enzymes. Consequently, these various types of fibres differ in intrinsic speed of contraction, power output and endurance. Such phenotypic diversity has been attributed to the myo-regulatory function of the motor nerve supply. According to this hypothesis, muscle fibres are considered to be "plastic", and fibre types interconvertible according to the pattern of impulses received from the nerve (1,2).

During myogenesis, myotubes are uniformly slow contracting and synthesize embryonic and foetal myosins before expressing adult fibre characteristics. In view of the profound influence motor nerves have on mature muscle fibres, it has generally been considered, since the work of Buller, Eccles and Eccles (3,4) that the emergence of muscle fibre heterogeneity during myogenesis is also brought about by the action of nerves on a common, undifferentiated myotube.

The experiments described in this paper were done to test the neural regulatory hypothesis for fibre type diversity. The jaw muscles of carnivores contain superfast muscle fibres which express a unique myosin not found in limb muscles. If the neural regulatory hypothesis is valid for these muscles, jaw muscles regenerating in limb muscle

beds should express limb muscle myosins and vice versa. During limb muscle development, muscle fibres are polyneuronally innervated. Emerging myotubes would be expected to co-express a mixture of adult myosins. The results of these experiments obtained with immunocytochemical techniques using monoclonal and polyclonal anti-myosin heavy chain antibodies do not confirm these expectations. They reveal two hierarchically distinct levels of myogenic influences affecting fibre phenotype. A hierarchical classification is proposed in which jaw and limb muscles belong to different allotypes which define their phenotypic options. Within each allotype, myogenically distinct isotypes emerge during development.

RESULTS

Nerve Independent Intrinsic Differences Between Cat Jaw and Limb Muscles

Strips of posterior temporalis muscle, a homogeneous superfast muscle, were treated with Marcaine to destroy mature muscle fibres and transplanted into limb muscle beds for regeneration and reinnervation by the host nerve (5). Early regenerates in the bed of either the fast extensor digitorum longus (EDL) or the slow soleus muscle react with antibodies against the heavy chain of foetal, slow or superfast myosins, but not with antibodies against fast myosin. In the long-term, regenerates innervated by the EDL nerve express only superfast myosin whereas in the regenerates innervated by the soleus nerve most fibres react only with the anti-slow myosin antibody, while some fibres react only against superfast myosin even after 213 days. In contrast, EDL and soleus muscles regenerating in their own beds express foetal, slow and fast myosins, but do not express superfast myosin. The isometric contraction times of the various types of regenerates reflect the types of myosin synthesized.

The ability of the regenerating superfast muscle to express the superfast myosin is independent of the nerve (6). This is shown in experiments in which reinnervation of the transplant in the EDL bed is prevented by cutting the common peroneal nerve and reflecting it back into the thigh. In these denervated beds the early temporalis regenerates are indistinguishable from innervated regenerates in expressing superfast myosin in addition to foetal and slow myosins.

Intrinsic differences between jaw and limb muscle cells

have also been shown by the transplantation of limb muscles into the jaw muscle bed. Jaw muscle beds are less satisfactory from the point of view of defining the innervation of the regenerates. Limb muscle strips were transplanted into the anterior temporalis bed after partial excision of this muscle. Fig. 1 shows the results of an EDL regenerated in the anterior temporalis muscle bed for 12 weeks. Staining for superfast (Fig. 1a) and foetal (Fig. 1b) myosins is negative, whereas nearly all fibres stain for fast myosin (Fig. 1d), and some fibres also stain for slow myosin (Fig. 1c). Although innervation of these fibres is not specifically demonstrated, their large size and the absence of staining for foetal myosin suggests that they are innervated.

It is concluded that jaw and limb muscle cells are two distinct types of muscle cells, each having a distinct repertoire for the expression of adult isomyosins, and that the particular isomyosin expressed can be modulated by the nerve.

Heterogeneity of Primary Fibres in Developing Limb Muscles

The postnatal development and the effects of neonatal denervation on muscle fibres in the EDL and tibialis anterior (TA) muscles of the rat were studied immunocytochemically using monoclonal antibodies against myosin heavy chains. Three types of primary myotubes (fast, intermediate and slow) with distinct topographic distributions can be distinguished perinatally. All primaries synthesize slow myosin initially, but in fast and intermediate primaries, slow myosin is no longer detectable in the neonatal period and at 2 weeks of age respectively. The fast primaries are localized principally in a superficial strip of the TA (Fig. 2B) where in the matured muscle slow fibres are absent. Slow primaries are located deep in the muscle while intermediate primaries lie in between. The distribution of slow and intermediate primaries at birth is shown in Fig. 2A.

Following neonatal denervation, the slow and intermediate primaries still express slow myosin, whereas the fast primaries do not stain for slow myosin. At three weeks after denervation, slow primaries are hypertrophic and intermediate primaries are atrophic, both staining with anti-slow antibody. The topographic distribution of these primaries is shown in Fig. 2C. These results show that the three different types of primary myotubes respond

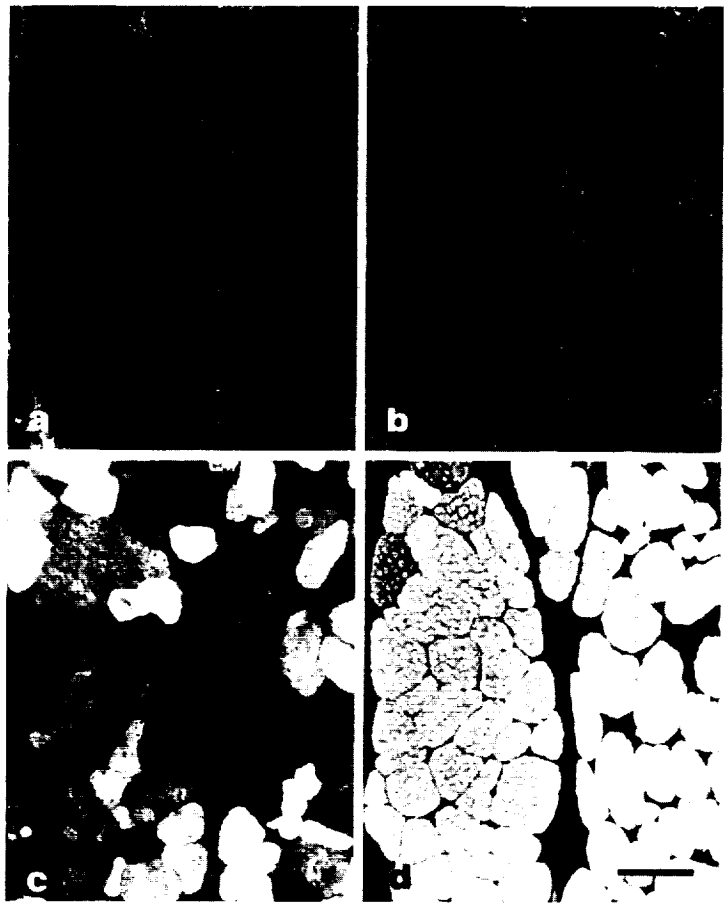

Fig. 1. Fluorescence photomicrographs of serial sections of cat extensor digitorum longus muscle regenerated in the anterior temporalis muscle bed for 12 weeks stained for superfast (a), foetal (b), slow (c), and fast/foetal (d) myosin heavy chains. The scale represents 100μm.

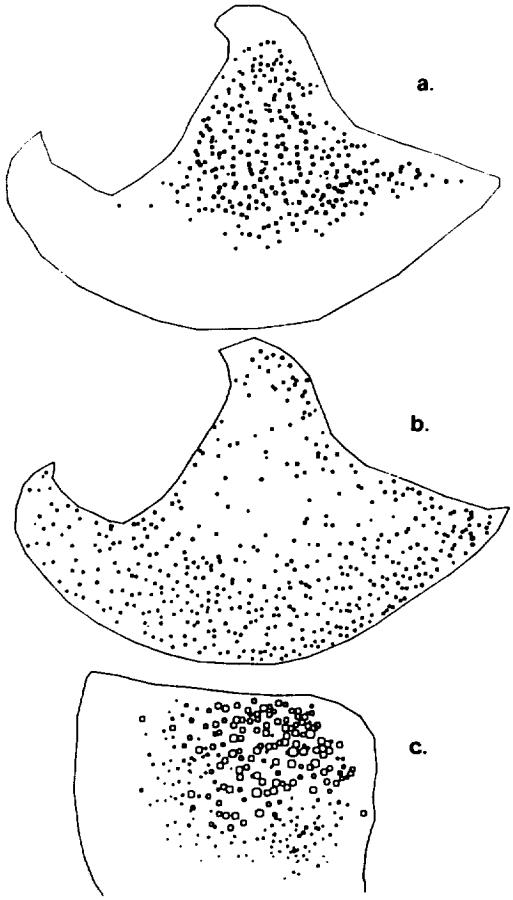

Fig. 2. Distribution of fibres which stain with anti-slow myosin heavy chain antibody in rat tibialis anterior (TA) muscle at birth (A, B) and TA three weeks after neonatal denervation (C). Fibres which stained strongly (slow and intermediate primaries) and those that stained faintly in the neonatal TA (fast primaries) are shown in (A) and (B) respectively. Note the hypertrophy of slow primaries and atrophy of intermediate primaries following denervation (C).

differently to denervation.

Heterogeneity of Secondary Fibres in Developing Limb Muscles

Immunocytochemical analysis of neonatal cat EDL and soleus muscles (7) has revealed that there are at least two distinct classes of secondary fibres. Both classes initially stain strongly for embryonic/foetal myosins. In one of these classes, developmental myosins are replaced by fast myosin. These fast secondaries do not stain for slow myosin nor react with anti-superfast myosin antibody at any stage. The other class of secondaries, the slow secondaries, are prevalent in the slow soleus muscle. These fibres acquired staining for slow myosin but not for fast or superfast myosins.

The vast majority of neonatal secondary myotubes in rat EDL and TA muscles are fast secondaries and stain with an anti-foetal/fast-red myosin antibody. These myotubes diverge at 9 days into a superficial fast-white region and a deep fast-red region. The majority of superficial secondaries no longer stain for foetal/fast-red myosins, presumably expressing fast-white myosin, whereas secondaries located in the region occupied by slow primaries predominantly express fast-red myosin. This topographical distribution of the two classes of secondaries is present in both the EDL and TA muscles, but is more conspicuous in the latter.

Following neonatal denervation in the rat, the divergence of fast-red and fast-white fibres in the EDL and TA muscles is not abolished, but delayed till three weeks post-operatively, suggesting that this divergence is neurally independent.

Effects of Denervation on Slow Primaries in Adult Rats

Immunocytochemical analyses of rat limb skeletal muscle fibres using specific anti-myosin antibodies have revealed that post-denervation changes of muscle fibres cannot be predicted by the fibre phenotype (8). The number of slow fibres in the EDL of a three month old rat is about half the number of slow primaries seen during development. Upon denervation of this muscle, the number of slow fibres increases to match the number of slow primaries at birth. These fibres hypertrophy while other fibres suffer denervation atrophy. These observations suggest that about half

of the slow primaries in mature rats undergo fibre type transformation into phenotypically fast fibres. Upon denervation, all slow primaries express slow myosin and hypertrophy, just as they do after neonatal denervation, irrespective of their phenotype at the time of denervation.

Heterogeneity of Primary and Secondary Fibres in Developing Cat Jaw Muscles

There are two phenotypes in jaw-closing muscle fibres in the cat: superfast and slow. In the posterior temporalis muscle of the mature animal, all fibres are superfast. During late foetal life, sections of this muscle stained for slow myosin appear very similar to those of fast limb muscle: slow staining primary fibres surrounded by rosettes of secondary fibres. Later, both primary and secondary fibres synthesize superfast myosin and the slow myosin in primary fibres is withdrawn (9). Primary fibres in the posterior temporalis are therefore analogous to fast primaries in limb muscles, and may be termed superfast primaries. Slow fibres are present in the anterior temporalis and the masseter muscles of adult cats, and these developmentally are derived from both primary and secondary fibres. The jaw slow primary fibres are analogous to slow primaries in limb muscles in which slow myosin synthesis persists to adult life. The jaw slow secondaries appear in early postnatal life, and some of these fibres stain also for superfast myosin during this period. At no time during the development of cat jaw muscle fibres does any fibre stain for fast myosin.

DISCUSSION

The results of these experiments reveal that the neural regulatory hypothesis cannot account for the difference between limb and jaw muscles. Each type of muscle has a specific repertoire for myosin gene expression, the limb muscles express slow, fast-red and fast-white myosins while jaw muscles express slow and superfast myosins. The ability to express these myosins is intrinsic to the muscle type, and can occur during regeneration in the absence of innervation. Innervation by limb nerves does not induce jaw regenerates to express fast myosins, nor does innervation by jaw nerve fibres lead to the expression of superfast myosin in limb regenerates. However, the specific form of myosin expressed by jaw or limb muscles is subject

to neural regulation within the constraints of the repertoire. The limited repertoires for myosin gene expression for jaw and limb muscles is also seen during developmental myogenesis.

It is useful to introduce the term allotype to describe different classes of skeletal muscle fibres with distinct intrinsic properties such as limb and jaw muscles. Jaw and limb allotypes probably arise from distinct lineages of myoblasts committed to differentiate along different paths. Extraocular muscles, which are isometrically faster than limb and jaw muscles (10) and which express a unique myosin heavy chain (11) may be another skeletal muscle allotype.

Immunocytochemical analyses of developing limb and jaw muscles reveal considerable heterogeneity in the pattern of myosin gene expression in both primary and secondary fibres. Such heterogeneity may be due to some extrinsic influence, such as innervation, acting upon a homogeneous population of myotubes. Alternatively, the myotubes may be intrinsically heterogeneous, being preprogrammed to express different types of myosin during subsequent development.

Evidence against the suggestion that fibre type diversity emerges as a result of neural regulation is the observation that divergence of fast and slow primaries is already apparent prenatally (12) whereas the impulse patterns of developing fast and slow motoneurons in the neonatal rat are very similar; differences emerge only at 3 weeks postnatally (13). Furthermore, the occurrence of polyneuronal innervation of muscle fibres (14) in the early postnatal period also argues against the neural regulatory hypothesis.

In support of the notion that myotubes are intrinsically heterogeneous may be cited the observations that clonal colonies of early myoblasts in chicken (15) and human embryos (16) are not homogeneous with respect to nutrient requirements and colony morphology. Miller and Stockdale (17) have isolated three types of clones from early chicken myoblasts which express fast, slow or a mixture of both myosins. These clones provide a nerve-independent mechanism for the generation of different muscle fibre types during myogenesis (18).

We propose that the emergence of diverse primary fibres in mammalian limb and jaw muscles is due to various lineages of myoblasts with intrinsicallydifferent properties. The characteristic responses to neonatal denervation of the three types of primaries in the rat limb clearly reveal their differences, the most spectacular feature of which being the

hypertrophy of slow primaries. This property of slow primaries is retained in the adult even though some of the slow primaries had apparently undergone, through the neural regulatory influence, a phenotypic change to become fast fibres. Thus, the hypertrophic response of denervated adult muscle fibres cannot be predicted on the basis of fibre phenotype, but can be so predicted according to the developmental origin of the fibres. Hence it is important to classify muscle fibres in accordance with their developmental origin in addition to their allotype and their phenotype. We propose to use the term isotype in this context. Thus there are at least three isotypes (slow, intermediate and fast) of primary fibres in limb muscles and two isotypes (superfast and slow) of primary fibres in jaw muscle.

The emergence of phenotypic characteristics of secondary fibres occurs relatively late during myogenesis, making it possible for neural regulatory mechanisms to have an impact on it. However, the possibility of there being various isotypes of secondary fibres cannot be discounted. Our neonatal denervation study shows that the divergence of fast-red and fast-white fibres is neurally independent, raising the possibility of the existence of two distinct isotypes of fast secondary myotubes. An interesting alternative mechanism for generating different phenotypes of secondary fibres is for primary fibres to influence the phenotype of the secondary fibres associated with them. The existence of gap junctions between primary and secondary fibres is well established (19). These junctions may provide the physical basis for the postulated myogenic influence on secondary fibres. The co-localization of slow primaries and fast-red fibres in the deep region of TA is consistent with the notion that slow primaries induce the expression of the fast-red phenotype in secondary myotubes associated with them.

The various myogenic and neurogenic influences on the phenotypic expression of myosin genes during myogenesis may now be summarized. The allotype defines the various phenotypic options available: superfast and slow myosins for the jaw allotype and fast-red, fast-white and slow for the limb allotype. Very early in myogenesis, diverse isomyoblasts emerge within each allotype. These fuse to produce myotubes of corresponding isotypes, each destined to undergo a particular pattern of myosin gene expression within the options defined by the allotype. Innervation may only play a trophic or permissive role on myogenesis up to this point.

Neural regulatory influences may operate after polyneuronal innervation has been eliminated and phasic and tonic nerve impulse patterns established. These influences may change the fibre phenotype within the range of options defined by the allotype, but do not alter the fibre isotype, nor transform the fibre allotype.

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COMPARATIVE ASPECTS OF HEMATOLOGICAL RESPONSES IN ANIMAL AND HUMAN MODELS IN SIMULATIONS OF WEIGHTLESSNESS AND SPACE FLIGHT

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This paper reviews some human and animal responses to space flight as well as in control models in simulations of weightlessness. Astronauts after space flight have been found to have a decreased red blood cell mass and plasma volume. The reason for these changes is unknown but appears to be caused primarily by a decrease in the need of red blood cells in the weightless condition. Similar though more moderate changes have been found in human subjects subjected to prolonged bed rest or water immersion. What happens to the red cell mass of laboratory rats flown in microgravity is not known but rats have shown an increase in the rate of random red cell loss in flight suggesting a probable decrease. Rat models subjected to either head-down suspension or restraint alone have shown a decrease in red blood cell masses and a decrease in their plasma volume.

I. Introduction

Numerous studies have shown that astronauts after flights have a reduction in their ^{51}Cr red cell mass and ^{125}I HSA plasma volume and consequently a decreased blood volume (6,26). Similar changes have occurred in human subjects in simulations of weightlessness produced by bed rest with or without head-down tilt (1,8,10,13, 14,17,19,24). Far fewer studies have been carried out on animals flown in microgravity and it is not known whether the laboratory rat is a valid model for the changes which occur in humans during space flight (4,5,9,11,12,15,16,21-23). However, rats subjected to either antiorthostatic or orthostatic hypokinesia/hypodynamia exhibit some of the same changes in red cell mass and plasma volume found in astronauts after space flight (2,3).

We have compared the hematological changes found after space flight with changes found in simulated weightlessness. Because of limitations in space, we concentrate on the studies of human astronauts and their simulated controls on Spacelab 1 (SL-1). The focus on animal studies was on the results from animals flown on Spacelab 3 (SL-3) together with ground-based simulation experiments. The results of these studies have been previously published separately (2,3,11,12, 14). Results of other investigators will be discussed as space permits.

II. Human Studies

Although some of the red blood cell changes found in early flights of NASA spacecraft were undoubtedly due to hyperoxic damage to red blood cells caused by the utilization of an increased partial pressure of oxygen, the Russian experience plus the results of Skylab and shuttle studies effectively rule out hyperoxia as a cause of the decrease in red cell mass (6,26).

The results of Skylab reticulocyte studies pointed towards a decreased production of red blood cells. This was investigated in personnel who flew on SL-1 and in simulation subjects who were selected on the basis of similarity of age, weight, sex (male), physical condition and overall health status (14). During the simulation of the inflight period, the control subjects were placed at -6° head-down bed rest for a period equal to the flight period. The following table shows the changes in red cell mass and plasma volume in SL-1 flight personnel, and control subjects.

Table 1. Percent decrease in red cell mass (RCM) and plasma volume (PV)

	RCM		PV		
	Mean	S.E.	Mean	S.E.	N
SL-1, 10 days	9.3*	1.6	6.0	4.3	4
Bedrest, 10 days	4.6*	1.2	5.4	4.4	5

*Significantly ($p<.05$) different from preflight measurement.

The changes in reticulocyte numbers are shown in Table 2.

Table 2. Reticulocyte numbers x 10^9/L

	Preflight	MD-1	L+0	L+8
Flight	64±5	49±15	24±8*	48±5
Bedrest	35±6	36±4	38±6	32±9

*Significantly different ($p<.05$) from preflight measurement. MD = mission day; L = landing day.

As shown in Table 2 the reticulocyte number decreased in the astronauts, indicating a probable decrease in production of red blood cells. However, this was certainly not complete and as shown in Table 3 incorporation of radioactive iron injected preflight was quite similar in control and flight subjects. The post-flight decrease in the calculated red blood cell iron incorporation suggests an increased red blood cell production in crew members.

Table 3. RBC iron incorporation SL-1 and bedrest simulation (% in RBC)

	MD-1	MD-7	L+0	L+1	L+8	L+12
Flight	19	85	88	93	85	86
Bedrest	21	86	91	92	91	92

MD = mission day; L = landing day.

Also mitigating a complete shutdown of bone marrow production is the fact that the levels of serum iron and iron-binding capacity were unchanged. This also shows that iron stores are replete. Serum ferritin is a measure of iron stores and on SL-1, as shown in Table 4, there were significant increases seen on MD-7, L+0, and L+1. This could indicate that the iron from red blood cells lost early in flight was being

reutilized and also that the inflight loss of red blood cells was not the result of external hemorrhage.

Table 4. Ferritin changes SL-1 and bedrest simulation (percent change).

	MD-1	MD-7	L+0	L+1	L+8	L+12
SL-1	1.5	53*	62*	63*	1.9	-15
Bedrest	1.3	-13.9	-11.6	-11.9	-34.2*	-33.0*

*Significantly different ($p<.05$) from preflight measurements. MD = mission day; L = landing day.

The other major cause for a loss of red blood cell mass would be increased red blood cell destruction. However, radioactive tracer studies on SL-1 crew members showed that the percentage of red blood cells remaining at 8 days was the same as it was for simulation subjects and was normal. Intravascular hemolysis should lead to decreased serum haptoglobin, but haptoglobin increased slightly but not significantly.

III. Animal Studies

Simulation studies for SL-3 flight animal studies were carried out using rats suspended in a jacket and harness arrangement. In the first study the head-down angle was approximately 20° and by use of fore limbs the rats were able to move through 360° (2).

The results in the suspended rats showed:

A. Reduction in red blood cell mass,
B. Suppression of erythropoiesis,
C. A transient increase in hematocrit due to a reduction in plasma volume,
D. A post-exposure hematocrit decrease,
E. A weight loss (or failure to thrive),
F. A reduction in food and water consumption.

Similar results are observed in man so at least in a gross sense, the rat "model" seemed to reproduce many of the known hematological effects found during and after space flight.

The studies were expanded to evaluate the effect of restraint alone as opposed to head-down tilt and many of the same changes were found (3). Changes in red blood cell clearance were thought to be unique to the head-down posture. This is currently being reevaluated and preliminary results have shown no change in red blood cells remaining in the circulating red blood cell mass. Thus, survival was normal (R. Nachman, unpublished observations).

While the changes in red cell parameters have been conclusively shown in astronauts, to our knowledge no isotope studies of red cell mass have been performed on rats flown in space so it is not known if the rat is indeed a potential model for "space anemia."

On SL-3, 24 rats were flown on the 8-day flight. The hematology studies performed after the flight showed (11,12):

A. Hematocrits, red blood cell counts, and hemoglobin determinations were increased in flight animals. The number of reticulocytes were slightly decreased in the large rats and slightly increased in small rats but the differences were not significant.

B. There were no significant differences from control animals in spleen cell differentials or erythropoietin determinations for control and flight animals.

C. The bone marrow cells of flight animals demonstrated an increased sensitivity to erythropoietin when grown in methylcellulose cultures.

IV. Discussion

A comparison of the results of changes in red blood cell parameters in the human and rat studies are shown in Tables 5 and 6.

Table 5. Human studies

	SL-1			Bedrest Simulation 6° Head Down		
	L+0	L+8	L+13	L+0	L+8	L+13
RCM	↓*	↓	--	↓*	↓*	--
Plasma Vol	↓	↑	--	↓	NC	--
Blood Vol	↓*	NC	--	↓	↓	--
HCT	↓	↓*	↓*	↑	↓	--
RBCC	↓	↓*	↓*	↑	↓	↓
Hgb	↑*	↓*	↓*	↑	↓	↓
MCV	↑	↑	↑	NC	NC	NC
MCH	↑*	NC	NC	NC	NC	NC
MCHC	↑*	↓	↓	↑	↑	↑*
RBC Shape						
Discocyte %	↓	↓	NC	--	--	--
Echinocyte %	↑	↑*	↑	--	--	--
BM Ery.	--	--	--	--	--	--
Retics	↓	--	NC	↑	↓	↑
Fe Inc.	88%	--	--	91%	--	--
Serum Fe	NC	↓	↓	↓	↓	↓
Ferritin	↑*	NC	↓	↓	↓*	↓*
Haptoglobin	↑	↑	↑	↑	NC	NC
Epo	↓	↓	↓	↑	NC	NC
RBC Surv.	NC	--	--	NC	--	--

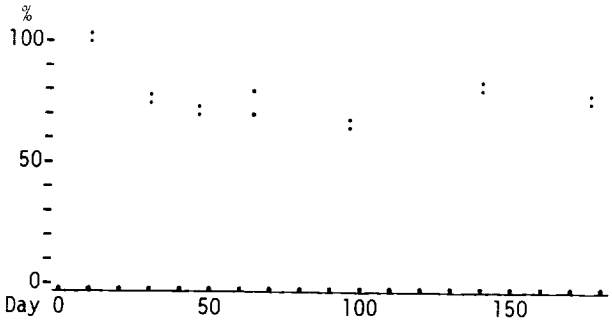
NC = Unchanged
* = Sig. <0.05.

Table 6. Rat studies

	SL-3 L+0		Head Down Suspended Rats		
	Sm. Rats	Lg. Rats	L+0	L+8	L+28
RCM	--	--	↓*	↓	NC
Plasma Vol	--	--	↓*	↓	NC
Blood Vol	--	--	↓*	↓*	NC
Hct	↑*	↑	↓*	↓*	↑
RBCC	↑*	↑	NC	↓	--
Hgb	↑*	↑	↓*	↓	--
MCV	↓	↓	↓*	↓	
MCH	↓	NC	--	--	--
MCHC	↓	↑	--	--	--
RBC Shape					
Discocytes	--	--	NC	NC	NC
Echinocytes	--	--	NC	NC	NC
Retics	↑	↓	↓*	↑	↓
BM Ery.	↓	↓	NC	NC	NC
Fe Inc.	--	--	↓*	NC	NC
RBC Surv.	--	--	↓*	--	--
Epo	↓	--	↑	--	--

NC = not changed from control.
-- = not performed.
* = Sig. <0.05

U.S.S.R. studies have also shown a decrease in hemoglobin mass of cosmanauts as shown in Figure 1.



Redrawn from Balakovskii et al. (1).
X axis, duration of (days).
Y axis, hemoglobin mass (% of base value).

The loss appears to level off so that after flights of 140 and 175 days mean decreases in hemoglobin mass (CO method) of -16 and -18 percent were found. Simulations by means of bedrest or immersion hypokinesia have shown a loss of red cell mass and plasma volume (10,13,14,17,20,24). The results of one study are shown in Table 7.

Table 7. Studies involving 30-day hypokinesia.

	HH[1]		AH[3]		Control	
	Hgb M[4] g/m^2	Retic[5] %	Hgb M g/m^2	Retic %	Hgb M g/m^2	Retic %
Before	432	5.7	420	6.9	418	7.8
After 30 days	343[6]		350[6]		---	
14-17 d read pt.	---	13.0[7]	404	12.6[7]	420	9.4

From Balakovskii et al. (1).
[1] = horizontal hypokinesia
[3] = antiorthostatic hypokinesia
[4] = hemoglobin mass
[5] = reticulocyte count
[6] = p = <0.02
[7] = p = <0.001

It is of interest that Kakurin et al. (7) found that antiorthostatic hypokinesia at -12° reproduced more closely the physiological responses shown in space crew members than did horizontal bed rest alone or head-down tilt at other angles.

In flight animals flown in space on Cosmos flights, Gazenko and Ilyin et al. found no differences in the values of hemoglobin, hematocrit and red blood cell counts (4,5). Gazenko et al. (4) and Leon et al. (16) found evidence for a hemolytic component in flight animals. However, in later studies, Leon et al. (15) found that when the animals were centrifuged in flight to produce the effect of gravity, the hemolysis was prevented indicating that the hemolysis results from the lack of gravity rather than other factors in the flight enfironment. Similar to the results of SL-3 rats, LeBlanc (unpublished observations) found that in flight animals there was an early post-flight significant increase in red blood cell counts, hemoglobin, hematocrit and mean corpuscular volume. Some observers have found a decrease in bone marrow red blood cell precursors in flight animals (4,5,21,23) though no changes were found in the SL-3 rats. It is apparent that findings for animal studies have varied from one study to the other and point up the need for isotopic studies of plasma volume and red cell mass to determine if the changes mimic those observed in humans participating in microgravity flights.

We still do not know the cause of the reduction in red blood cell mass in astronauts 19 years after the first description of this phenomena by Fischer, Johnson and Berry (6) and whether the rat is a proper model for "space anemia." Lists of possible causes for the anemia have been published (6,26). The anemia may be caused and maintained by decreased production of red blood cells which could be multifactorial. Some of the accumulated data suggests that after an initial decrease in the circulating red cells, the "erythrostat" appears to be reset at a lower level due to a decreased demand brought on by weightlessness. This is analogous to the atrophy of disuse seen in muscles and other tissues and the body's setting of ideal points for organ weights. The need to perform isotope studies of red cell mass and plasma volume to determine if these values decrease as do those of human astronauts is inferred from the animal studies. The needs for future studies were pointed out by a working group of the life sciences research office of the Federation of American Societies of Experimental Biology and are given in Table 8 (25).

Table 8. Baseline data for analysis of erythrokinetics of space flights*

	Settings and Subjects			
	Ground-Based**		Inflight***	
Parameter	Animal	Human	Animal	Human
Red cell count	+	+	+	+
Hemoglobin	+	+	+	+
Hematocrit	+	+	+	+
Red cell mass	+	+	+	+
Blood volume	+	+	+	+
Plasma volume	+	+	+	+
Reticulocyte count	+	+	+	+
Erythropoietin	+	+	+	+
Plasma or serum haptoglobin	+	+	+	+
Platelets	+	+	+	+
Red cell shape	+	+	+	+
Red cell size	+	+	+	+
Blood P_{50}	+	+	+	+
Blood PCO_2	+	+	+	+
Red cell ATP	+	+	+	+
Red cell 2,3-DPG	+	+	+	+
Red cell sodium	+	+	+	+
Skin petechiae	-	-	+	+
Subcutaneous, subserosal oozing of RBC	-	-	+	-
Bone marrow smear	+	-	+	-

*When feasible, measure sequentially for temporal aspects.
**Examples: Biological laboratories, hospitals, space simulation facilities (bed rest, water immersion, etc.), spacecraft simulators.
***Include pre-, in-, and postflight phases.

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